PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2 5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day month year) 02 May 2001 (02.05.01)	
International application No. PCT GB00 03182	**Applicant's or agent's file reference** NSM PIB 40875
International filing date (day month year) 16 August 2000 (16.08.00)	**Priority date** (day month year) 19 August 1999 (19.08.99)
Applicant COCKERILL, Gillian et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 March 2001 (07.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Zakaria EL KHODARY

Telephone No.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
NSM/PIB/40875		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/03182	16/08/2000	19/08/1999
Applicant		
QUEEN MARY AND WESTFIELD COLLEGE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

HIGH DENSITY LIPOPROTEIN AGAINST ORGAN DYSFUNCTION FOLLOWING HAEMORRHAGIC SHOCK

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P[redacted]B 00/03182

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0780128 A	25-06-1997	DE 19547648 A	26-06-1997
		JP 9188658 A	22-07-1997
		US 5780592 A	14-07-1998
JP 10025248 A	27-01-1998	NONE	
WO 9706822 A	27-02-1997	AU 6773596 A	12-03-1997
		CA 2229140 A	27-02-1997
		CZ 9800454 A	13-01-1999
		EP 0868197 A	07-10-1998
		HU 9901673 A	30-08-1999
		JP 10510553 T	13-10-1998
		PL 325012 A	06-07-1998

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]B 00/03182

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61P39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 780 128 A (HOECHST AG) 25 June 1997 (1997-06-25) *see in particular claims 1,4,5,9; page 3, lines 4-9; example 5 * ---	1-6
A	PATENT ABSTRACTS OF JAPAN vol. 1998, no. 05, 30 April 1998 (1998-04-30) & JP 10 025248 A (CHEMO SERO THERAPEUT RES INST), 27 January 1998 (1998-01-27) abstract ---	1-6
A	WO 97 06822 A (PROTEIN DESIGN LABS INC ;BOEHRINGER MANNHEIM GMBH (DE); HASELBECK) 27 February 1997 (1997-02-27) *see in particular page 1, line 5 - page page 3, line 12; claims 1,10,22* --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	18/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Isert, B

1

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]B 00/03182

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
A	COCKERILL ET AL.: "High-density lipoproteins differentially modulate cytokine-induced expression of E-selectin and cyclooxygenase-2 " ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, vol. 19, April 1999 (1999-04), pages 910-917, XP000944093 *see the abstract; p.915, last paragraph of the discussion * -----	1-6

1

PATENT COOPERATION TREATY

PCT

RECD 27 JUN 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
NSM/PIB/40875		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03182	16/08/2000	19/08/1999
International Patent Classification (IPC) or national classification and IPC A61K38/17		
Applicant QUEEN MARY AND WESTFIELD COLLEGE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/03/2001	25.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Isert, B Telephone No. +49 89 2399 8691

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03182

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1/15-15/15 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 6.

because:

☒ the said international application, or the said claims Nos. 6 (for industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-6

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-6
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-5
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION III

1). Claim 6 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

SECTION V:

2). The following documents (D) cited in the International search report are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 = EP-A- 780128
D2 = JP-A- 10025248 (as PAJ abstract)
D3 = WO-A-97 06822
D4 = Arterioscler. Thrombos. Vascul. Biol. , April 1999, 19:910-917

Unless indicated otherwise reference is made to the relevant passages emphasized in the search report.

3). Novelty:

The subject-matter of claims 1-6 is novel over D1-D4:

D1 relates to the use of HDL in combination with crotonic acid derivatives for treating various diseases, inter alia acute transplant rejection. The efficacy of HDL on this respect has been shown (example 5).

D2 describes the use of Apolipoprotein AI for treating restenosis after angioplasty

D3 discloses a method for reducing the probability of incidence of organ

failure after a polytraumatic event, comprising administering an anti-selectin antibody.

D4 shows that HDL induces an antiinflammatory phenotype in cytokine-induced ECs, synergizing with cytokine to induce elevation of Cox-2 in addition to inhibiting adhesion molecule expression.

4). Inventive step:

The subject-matter of claims 1-6 is considered inventive:

The application relates to the treatment of organ dysfunction resulting from haemorrhagic shock. It has inter alia been shown in the working example 1 that HDL is protective in rats suffering from said condition.

Document D3 is considered closest prior art, which discloses the reduction of post-traumatic mortality due to hemorrhagic-traumatic shock failure after administration of anti-selectin antibodies to baboons (example 2). Selectins are adhesion promoters known to be involved in inflammatory reactions (D3, page 4, lines 9-19).
In contrast to D3 the present application suggests the use of HDL lipoproteins to treat organ dysfunction resulting from hemorrhagic shock. It is known from D4 that HDL induces an antiinflammatory phenotype in cytokine-induced endothelial cells. However, when combing D3 and D4 a skilled man could not expect that HDL would have the therapeutic effect claimed.

5). Industrial applicability

For the assessment of the present claim 6 on the question whether it is are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a

known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The claims 1-5 relate to a new use of known pharmaceutical products which are considered industrially applicable under Article 33(4) PCT.

SECTION VII

6). Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

SECTION VIII

7). The abbreviations "HDL", "apo A-I" and "apo A-II" used in claims 3-5 should be replaced by the complete chemical name (cf. page 4 of the description).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13939 A1

(51) International Patent Classification[7]: A61K 38/17, A61P 39/00

(21) International Application Number: PCT/GB00/03182

(22) International Filing Date: 16 August 2000 (16.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9919713.9 19 August 1999 (19.08.1999) GB

(71) Applicant *(for all designated States except US)*: **QUEEN MARY AND WESTFIELD COLLEGE** [GB/GB]; University of London, Mile End Road, London EC1 4NS (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **COCKERILL, Gillian** [GB/GB]; 54 Southdown Avenue, London W7 2AF (GB). **MILLER, Norman** [GB/GB]; Flat 28, Lexington Apartments, 40 City Road, London EC1Y 2AN (GB). **THIEMERMANN, Christoph** [DE/GB]; Flat 42, Limehouse Cut, 46 Morris Road, London E14 6NQ (GB). **MACDONALD, Michelle** [GB/GB]; 116 Cumberland Road, Hanwell, London W7 2EB (GB).

(74) Agent: **MARLOW, Nicholas, Simon**; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/13939 A1

(54) Title: HIGH DENSITY LIPOPROTEIN AGAINST ORGAN DYSFUNCTION FOLLOWING HAEMORRHAGIC SHOCK

(57) Abstract: Use of high density lipoprotein and/or derivatives in the manufacture of a medicament for the prevention or treatment of organ dysfunction following ischaemia and reperfusion injury. In particular, the medicament may be for the treatment of end stage organ injury or failure.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13939 A1

(51) International Patent Classification⁷: A61K 38/17, A61P 39/00

(21) International Application Number: PCT/GB00/03182

(22) International Filing Date: 16 August 2000 (16.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9919713.9 19 August 1999 (19.08.1999) GB

(71) Applicant *(for all designated States except US)*: **QUEEN MARY AND WESTFIELD COLLEGE** [GB/GB]; University of London, Mile End Road, London EC1 4NS (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COCKERILL, Gillian** [GB/GB]; 54 Southdown Avenue, London W7 2AF (GB). **MILLER, Norman** [GB/GB]; Flat 28, Lexington Apartments, 40 City Road, London EC1Y 2AN (GB). **THIEMERMANN, Christoph** [DE/GB]; Flat 42, Limehouse Cut, 46 Morris Road, London E14 6NQ (GB). **MACDONALD, Michelle** [GB/GB]; 116 Cumberland Road, Hanwell, London W7 2EB (GB).

(74) Agent: **MARLOW, Nicholas, Simon**; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/13939 A1

(54) Title: HIGH DENSITY LIPOPROTEIN AGAINST ORGAN DYSFUNCTION FOLLOWING HAEMORRHAGIC SHOCK

(57) Abstract: Use of high density lipoprotein and/or derivatives in the manufacture of a medicament for the prevention or treatment of organ dysfunction following ischaemia and reperfusion injury. In particular, the medicament may be for the treatment of end stage organ injury or failure.

HIGH DENSITY LIPOPROTEIN AGAINST ORGAN DYSFUNCTION FOLLOWING HAEMORRHAGIC SHOCK

The present invention relates to the manufacture of medicaments for protecting against organ damage following haemorrhagic shock, using high-density lipoproteins (HDLs) and derivatives thereof. In particular, it relates to manufacture of medicaments for treatment and prevention of end-stage organ failure following haemorrhagic shock.

Many victims of sudden physical injury (for example, traffic accident victims) die because of end-stage organ failure. In patients with this condition, biochemical and biological changes (such as haemodynamic changes and microthrombus formation) occur in the blood and organs (such as liver and kidneys) due to shock and blood loss; this is a different action to "endotoxic" shock which arises due to bacterial infection. If end-stage organ failure is not halted or prevented, it will lead to permanent organ damage and death of the patient. There is a need for a pharmaceutical agent which can be administered as soon as possible after the physical injury, preferably at the site of the accident in order to prevent end stage organ failure, and which can also be used subsequently while transporting the victim from the accident site to casualty/hospital, and while the physical wounds are being treated.

High-density lipoproteins (HDLs) form a range of lipoprotein particles found in normal serum. Mature HDL particles are present in the form of a globular structure containing proteins and lipids. Within the outer layer of these

particles are the more polar lipids, phospholipids and free cholesterol, all having charged groups pointing outwards towards the aqueous environment. The more hydrophobic lipids, such as esterified cholesterol and triglycerides, reside in the core of the particle. Newly formed, or nascent, HDL particles lack the lipid core and are discoidal in shape. Protein components are embedded in the outer layer. The main protein component is apolipoprotein A-I (apo A-I), with smaller amounts of apo A-II, apo A-IV, apo CIII, apo D, apo E and apo J. Various other proteins reside on the HDL particle, such as lecithin-cholesterol acetyl transferase, PAF acetylhydrolase and paraoxonase.

The binding of activated leukocytes to the endothelium is the earliest observable cellular event in a number of acute and chronic inflammatory diseases. This binding is mediated by the expression of adhesion molecules on the surface of the endothelial cells which bind to corresponding molecules of similar function on leukocytes. Recently we have shown that pre-treatment of endothelial cells, *in vitro*, with HDL was able to inhibit the cytokine-induced expression of these adhesion molecules (Cockerill GW, Rye K-A, Gamble JR, Vadas MA, Barter PJ. Arterioscler Thromb. Vasc. Biol. 1995, 15: 1987-1994 1995, Cockerill GW Reed S.Int.Rev.Cytol: A survey of cell biology 1999). In addition, we have recently shown that HDL can inhibit cytokine-induced adhesion molecule expression in an acute inflammatory model in the pig (Cockerill et al., submitted 1999). The antiflammatory effects of HDL have thus been demonstrated in these models where cells/animals are pre-treated with lipoprotein.

End-stage organ failure following haemorrhagic shock results from the adhesion of polymorphonuclear leukocytes (PMNs) to the endothelium following their activation caused by ischaemia and reperfusion injury. We have now found that administration of HDL or derivatives thereof prevents end-stage organ failure following haemorrhagic shock.

According to the present invention high density lipoprotein and/or a derivative thereof is used in the manufacture of a medicament for the prevention or treatment of organ dysfunction following haemorrhagic shock.

Preferably, the medicament is for the treatment of end-stage organ injury or failure.

We have shown that, following haemorrhagic shock, HDL is able to perturb the damaging effects when given after the initial hypovolaemia has occurred. Our work suggests that at physiological levels (both *in vitro* and *in vivo*), native HDL particles are active in inhibiting the expression of adhesion proteins on endothelial cells. Prevention of expression of adhesion proteins on endothelial cells prevents binding of PMNs to the endothlium; thus administration of HDL prevents end-stage organ failure.

The high density lipoprotein may be the component of HDL that inhibits adhesion to the endothelial cells and subsequent activation of leukocytes or a derivative, molecule, homologue, or mimic thereof.

The inhibiting effect is not only present in venous endothelial cells but also on arterial endothelial cells and is independent of the nature of the lipid present in the HDL particles. Two effector molecules mediate the inhibitory effect namely apolipoprotein A-I (apo A-I) and apolipoprotein A-II (apo A-II) (Brouillette C.G. and Anatharamaiah G.M. Biochem.Biophys. Acta. 1256: 103-129. 1995; Massey J.B., Pownall H.J. Biochem.Biophys Acta. 999 : 111-120. 198); these two molecules have different efficacy of inhibition.

Preferably, the high density lipoprotein or derivative thereof is a peptide or protein derivative of the sequence of apo A-I or apo A-II, or a peptide or protein derivative functionally homologous to the active portions of apo A-I or apo A-II.

Preferably, the high density lipoprotein is reconstituted HDL. The term "reconstituted HDL" means HDL composed of a lipid or lipids in association with at least one of the apolipoproteins of HDL. The components may be derived, for example, from blood, or produced by recombinant technology.

The medicament may be administered to a patient in any conventional manner. Preferably the medicament is administered intravenously. Preferably, the medicament is administered using saline as a vehicle.

Preferably the medicament is provided in a portable dispenser, for example, for use at the site of an accident.

According to the invention in another aspect there is provided a method of treatment of organ dysfunction following haemorrhagic shock in a human patient which comprises the step of administering to a patient reconstituted high density lipoprotein and/or a derivative thereof in pharmaceutically acceptable form.

The present invention will now be illustrated with reference to the attached drawings in which :

FIGURE 1 shows alterations in mean arterial blood pressure (MAP) in rats subjected to (i) the surgical procedure without causing a haemorrhage and treated with vehicle for HDL (SHAM, open diamonds, saline, 3mg/kg i.v. bolus; n=9) or with recHDL (SHAM - recHDL open square, 80mg/kg i.v. bolus injection, n=9) or (ii) haemorrhage for 1.5 h and upon resuscitation with the shed blood, control rats were treated with the vehicle (HS open circles, saline 3ml/kg i.v. bolus; n=10), recHDL (HS - recHDL filled squares, 80mg/kg i.v. bolus injection, n=9) or nHDL (HS - nHDL open triangle, 80 mg/kg i.v. bolus injection) [nHDL = native HDL; rHDL or recHDL is reconstituted HDC];

FIGURE 1A shows a Table of heart rate in beats per minute(bpm) in all experimental groups studied before the haemorhhage -1.5 h and 1, 2, 3 and 4 h after resuscitation, Group 1 (SHAM): Rats were subjected to the surgical procedure without causing a haemorrhage and treated with a vehicle for HDL (saline, 1ml/kg i.v. bolus followed by an infusion of 1.5 ml/kg/h i.v.; n=9); Group 2 (SHAM - recHDL): Rats

were subjected to the same surgical procedure as Group 1 but treated with recHDLs (reconstituted HDLs) (80mg/kg i.v. bolus injection followed by an infusion of saline 1.5 ml/kg/h i.v. n=4); Group 3 (HS): Rats were subjected to a haemorrhage for 1.5 h and upon resuscitation with the shed blood were given an infusion of 1.5 ml/kg/h i.v., n=9); Group 4 HS-recHDL: rats were subjected to the same procedure as group 3 but treated with recHDLs (80mg/kg i.v. bolus injection followed by an infusion of saline 1.5 ml/kg/h i.v.; n=9); and Group 5 (HS-nHDL) : rats were treated in the same way as Group 4, but were given nHDLs instead of recHDLs prior to resuscitation.

FIGURE 2 shows plasma levels of (A) urea, (B) creatine, (C) AST, (D) ALT, (F) creatinine kinase (CK) and (E) lipase in rats subjected to the surgical procedure and experiment 2 described below;

FIGURE 3 shows the effect of HDL infusion on histological sections from lung, gut and kidney following haemorrhagic shock;

FIGURE 4A shows the effect of HDLs on myeloperoxidase (MPO) levels in the lung, as a measure of neutrophil activation;

FIGURE 4B shows the effect of HDLs on MPO levels in the kidney;

FIGURE 5A shows the effect of HDLs on the level of malonialdehyde (MAD) in the lung;

FIGURE 5B shows the effect of HDLs on the level of

- 7 -

malonialdehyde in the kidney;

FIGURE 6 shows a graph of mean fluorescence intensity (dependent on inhibition of E-selectin), as described below;

FIGURE 7A shows a graph of mean fluorescence intensity of HUVEC (veinous EC) against concentration of lipoproteins apo A-I and apo A-II for experiment 3, below; and

FIGURE 7B shows a graph of mean fluorescence intensity of HuAEC (arterial EC) against concentration of lipoprotein apo A-I and apo A-II for experiment 3, below.

As a demonstration of an embodiment of the invention, Experiment 1 describes the effects of human high-density lipoprotein (HDL) on the circulatory failure and multiple organ dysfunction injury (MODS) such as renal dysfunction and liver dysfunction caused by severe haemorrhage and resuscitation in the anesthetised rat. It should be noted that this is a model of end stage organ failure generated by haemorrhagic shock, and is not known to be a result of endotoxin release.

All experiments described herein were performed in adherence to the National Institute of Health guidelines on the use of experimental animals and in adherence to *Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*, published by HMSO, London.

Experiment 1

The study was carried out on Wistar rats (Tuck, Rayleigh, Essex, UK) weighing 250mg - 320g receiving a standard diet and water *ad libitum*. All animals were anaesthetised with thiopentone (120mg/kg i.p.) and anaesthesia was maintained by supplementary injections of thiopentone as required. The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37°C with a homeothermic blanket. The right femoral artery was catheterised and connected to a pressure transducer (Senso-Nor 840, Senso-Nor, Horten, Noway) for the measurement of phasic and mean arterial blood pressure (MAP) and heart rate (HR). These were displayed on a data acquisition system (MacLab 8e, ADI Instruments, Hasting, UK) installed on an Apple Macintosh computer. The right carotid artery was cannulated to bleed the animals (see hereafter). The jugular vein was cannulated for the administration of drugs. The bladder was also cannulated to facilitate urine flow and to prevent the possibility of development of post-renal failure. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilise for 15 mins. Then, blood was withdrawn from the catheter placed in the carotid artery in order to achieve a fall in MAP to 50mmHg within 10 mins. Thereafter, MAP was maintained at 50mmHg for a total period of 90 mins by either withdrawal (during the compensation period) or re-injection of blood. It should be noted that in these experiments, the amount of shed blood re-injected during the 90 min period of haemorrhage did not exceed 10% of the total amount of the blood withdrawn. The amount of blood withdrawn for rats subjected to haemorrhage and treated with vehicle (control group) was 7.0 ± 0.4ml

SD ; the amount of blood withdrawn from rats subjected to haemorrhage and treated with HDL (treatment group) was 7.0 ± 0.3ml (p>0.05). At 90 min after initiation of haemorrhage, the shed blood and an equivalent volume of Ringer lactic solution was re-injected into the animal.

The results are shown in Figures 1, 1A (Table 1), 2, 3 and 4.

FIGURE 2 shows plasma levels of (A) urea, (B) creatinine, (C) AST, (D) ALT, (F) creatinine kinase (CK) and (E) lipase in rats subject to (i) SHAM the surgical procedure without causing a haemorrhage and treated with vehicle for HDL (sham+saline,saline, 3ml/kg i.v. bolus i.v.; n=9) or with reconstituted HDL (sham +recHDL; 80mg/kg i.v. bolus injection, n=4, or (ii) haemorrage for 1.5.h and upon resuscitation with the shed blood, control rats were treated with the vehicle (hs (+ saline), saline 3ml/kg i.v. bolus n=9),reconstituted HDL (hs + recHDL, 80mg/kg i.v. bolus injection, n=9) or nHDL (HS - nHDLC). The administration regimes are detailed more fully in the text accompanying Fig.1A. Haemorrhage and resuscitation resulted in significant increases in the serum levels of urea and creatinine (n=9), as demonstrated by the increase in urea and creatine concentration between "sham" and hs (control). This renal dysfunction was attenuated by the administration (5 mins prior to resuscitation) of HDL (80mg/kg. i.v., n=9; p,0.05; ANOVA followed by Dunnett's test for multiple comparisons), as demonstrated by the concentration of urea and creatinine for "HS- recHDL" and HS-nHDL. Similarly,

HDL attenuated the liver injury (as monitored by a rise in serum AST and ALT - (C) and (D) - and the pancreatic injury (as measured by a rise in serum lipase - (E)) caused by haemorrhage and resuscitation. In contrast, recHDL and nHDL did not affect the delayed circulatory failure associated with haemorrhage and resuscitation (see Fig 1 and Fig.1A (Table 1)). Administration of recHDL to rats which were not subjected to haemorrhage did not result in the alterations in the serum levels of urea, creatinine, AST, ALT or lipase (n=4) and, hence, was not toxic at the dose used.

Organ dysfunction as measured by the degree of disruption of tissue architecture was reduced by treatment with HDLs.

Light microscopy. Organ (lung, kidney and small intestine) biopsies were taken at the end of Experiment 1 and fixed for one week in buffered formaldehyde solution (10% in PBS) at ambient temperature, dehydrated by graded ethanol, and embedded in Paraplast™ (Sherwood Medical, Mahwah, NJ, USA). Sections (7 µm thick) were deparafinised with xylene, and stained using either Van Gieson's Trichrome or Fuchsin, and examined using light microscopy (Dialux 22, Leitz).

Figure 3 shows photomicrographs of representative sections of lung (upper panels), small intestine (middled panels), and kidney (lower panels) from animals following haemorrhage for 90 min. and given vehicle (saline) along with shed blood at the beginning of resuscitation (A), nHDLCs (80mg/kg i.v. bolus injection) prior to resuscitation (B), or recHDLs (80mg/kg i.v. bolus injection prior to resuscitation (C).

Sections were visualised using Van Gelson's trichrome stain. Original magnification x 100.

When compared to organs obtained from sham-operated rats, which had not been subjected to haemorrhage and resuscitation (data not shown), Fig.3 demonstrates that the lung (top panels), small intestine (middle panels) and kidney (bottom panels), when subjected to haemorrhage and resuscitation (A), show oedema with loss of normal tissue structure. In contrast, organs from animals which had received nHDLs (B) or recHDLs(C) prior to resuscitation showed no significant change in morphology, and were not significantly different from the sham-operated rats (not shown).

HDLs reduced neutrophil infiltration into lungs and kidneys following haemorrhage and resuscitation.

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation was determined as previously described [Anderson, B.O., Brown, J.M, Shanley, P.F., Benserd, D.D., and Harken, A.H. (1991 Marginating neutrophils are reversibly adherent to normal lung endothelium. *Surgery* 109.51-61). Samples of lung and kidney were obtained and weighed. Each piece of tissue was homogenised in a solution containing 0.5% hexadecyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7.0) and centrifuged for 30 min at 20,000 x g at 4°C. An aliquot of the supernatant was the allowed to react with a solution of tetra-methyl-benzidine

1.6 mM and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. 1 mU of MPO activity was defined as the quantity of enzyme degrading 1 umol of peroxidase per min at 37°C, and was expressed in mU per mg of wet tissue.

Figure 4 : Graph showing the effect of HDLs on myeloperoxidase (MPO) levels in (A) lung or (B) kidney, as a measure of neutrophil activation. Values represent mean and SEM, n=9; *$p<0.05$ when compared to haemorrhagic shock (HS).

The ability of HDLs to inhibit the expression of adhesion molecules in this model is strongly supported by the serum data and histology. We further investigated the ability of HDLs to inhibit neutrophil infiltration by measuring the MPO levels in lung (Figure 4A) and kidney (Figure 4B). When compared to tissues obtained from sham-operated rats, rats subjected to haemorrhage and resuscitation (solid bars) show an increase in tissue MPO activity. This was reduced in rats which had been treated with either nHDLs or recHDLs prior to resuscitation with shed blood.

HDLs reduced malondialdehyde levels in lungs and kidneys following haemorrhage and resuscitation.

Determination of malondialdehyde. Malondialdehyde (MDA) levels in the lung and kidney were determined as an indicator of lipid peroxidation. Tissues were homogenised in 1.15% KCl solution. An aliquot (100 µl) of the

homogenate was added to a reaction mixture containing 200 µl 8.1% SDS, 1500 µl 20% acetic acid (pH 3.5 , 1500 µl 0.8% thiobarbituric acid and 700 µl distilled water. Samples were then boiled for 1 hour at 95°C and centrifuged 3,000 x g for 10 min. The absorbance of the supernatant was measured spectrophotometrically at 650 nm.

Figure 5 : Bar graph showing the effect of HDLs on the level of malondialdehyde (MAD) in lung (A) and (B) kidney, as a measure of the anti-oxidant properties of HDLs. Values represent mean and SEM, n=9; *p,0.05 when compared to haemorrhagic shock (HS).

HDLs have been shown to have anti-oxidant properties. The ability of HDLs to influence the MDA levels in lung (Figure 5A) and kidney (Figure 5B) was investigated. When compared to tissues obtained from sham-operated rats, rats subjects to haemorrhage and resuscitation (solid bars) showed a marked increase in tissue MDA activity. This activity was reduced in rats which had been treated with either nHDLs or recHDLs prior to resuscitation [HS - nHDL, HS - recHDL].

In conclusion, administration of recHDL and nHDL attenuates the renal, liver and pancreatic dysfunction following haemorrhagic shock.

Experiment 2

This experiment demonstrates which components of the effective therapeutic agent (HDL) are responsible for protection against haemorrhagic shock; in this experiment,

the ability of native HDL to inhibit cytokine-induced adhesion molecule expression on endothelial cells is compared with the ability of lipid-free apo A-I protein or protein-free lipid vesicles.

Cell culture : Human umbilical vein-derived endothelial cells (HUVEC) and human umbilical-derived artery endothelial cells (HUAEC) (Cockerill G.W, Meyer G, Noack L.Vadas MA, Gamble J.R. Lab.Invest.71 : 497-509.1994) were grown on gelatin-coated tissue culture flasks (Costar, High Wycombe, Bucks, UK) in medium 199 with Earle's salts (Gibco, Paisley, Scotland) supplemented with 20% foetal calf serum (FCS) (Gibco, Australia), 20mM HEPES, 2mM glutamine, 1mM sodium pyruvate, non-essential amino acids, penicillin and streptomycin, 50µg/ml endothelial cell growth supplement (Sigma, Dorset, UK) and 50µg/ml heparin (normal growth medium).

Flow cytometry : Cells were plated at 1 x 10^5 cells/30 mm well and incubated overnight at 37°C in 5% CO_2. Confluent monolayer cultures were then incubated (at concentrations indicated) for 19 hours with either, phosphate buffered saline (PBS) (vehicle control), native HDL, free apo A-I, phospholipid vesicles or discoidal HDL prepared with only apo A-I or apo A-II. Following these treatments the cells were washed gently in complete medium and TNFα (Miles Scientific) was added at 10ng/ml. Cells were then stained at 4 hours post stimulation in the following manner. Cells were washed in serum free medium and 200µl anti-E-selectin (1,2B6) was added for 1 hour at 37°C. Cells were then washed

in phosphate buffered saline (PBS) containing 5% newborn calf serum, 0.02% sodium azide, and 100ul of fluorescein isothiocyanate-conjugated secondary antibody added for 1 hour at 37°C. Cells were then washed three times in PBS and trypsinised, then centrifuged to form a pellet. The pellet was then resuspended in 2.5% formaldehyde in PBS containing 2% glucose and 0.02% azide and analyzed in a Coulter Epics Profile II flow cytometer.

Figure 6 shows that neither free apo A-I nor unilamellar vesicles (SUV) were able to inhibit TNFα-induced expression of E-selectin. This suggests that Apo A-I, the most abundant apolipoprotein in HDL, must be in a lipid particle in order to mediate inhibition of cytokine-induced adhesion molecule expression in endothelial cells. Both umbilical-derived venous (HUVEC) and arterial (HUAEC) endothelial cells were able to support the dose-dependent inhibition of cytokine-induced E-selectin expression by HDL (as shown by the decrease in intensity with increase of apo AI HDL from 0.25 t0 1.0mg/ml).

The therapeutic action of HDL is afforded by the apolipoprotein presented in a lipid particle, and cannot be mimicked by the whole protein alone, or lipid alone.

Experiment 3

To determine the efficacy of reconstituted discoidal HDLs particles containing either of the most abundant apolipoproteins (apo A-I or apo A-II), a comparison of the ability of these particles to inhibit cytokine-induced

adhesion molecule expression on HUVEC and HUAEC was carried out.

Cell culture : Human umbilical vein-derived endothelial cells (HUVEC) and human umbilical-derived artery endothelial cells (HUAEC) (Cockerill et al., 19994) were grown on gelatin-coated tissue culture flasks (Costar, High Wycombe, Bucks, UK) in medium 199 with Earle's salts (Gibco, Paisley, Scotland), supplemented with 20% foetal calf serum (FCS) (Gibco, Australia), 20 mM HEPES, 2mM glutamine, 1mM sodium pyruvate, nonessential amino acids, penicillin and streptomycin, 50µg/ml endothelial cell growth supplement (Sigma, Dorset, UK) and 50µg/ml heparin (normal growth medium).

Flow cytometry : Cells were plated at 1 x 10^5 cells/30 mm well and incubated overnight at 37°C in 5% CO_2. Confluent monolayer cultures were then incubated (at concentrations indicated) for 19 hours with either reconstituted discoidal HDL prepared with only apo A-I or apo A-II. Following these treatments the cells were washed gently in complete medium and TNFα (Miles Scientific) was added at 10ng/ml. Cells were then stained at 4 hours post stimulation in the following manner. Cells were washed in serum free medium and 200µl anti-E-selectin (1.2B6) was added for 1 hour at 37°C. Cells were then washed in phosphate buffered saline (PBS) containing 5% newborn calf serum, 0.02% sodium azide, and 200µl of fluorescein isothiocyanate-conjugated secondary antibody added for 1 hour at 37°C. Cells were then washed three time in PBS and trypsinised. The pellet was then

resuspended in 0.5% formaldehyde in PBS containing 2% glucose and 0.02% azide and analyzed in a Coulter Epics Profile II flow cytometer.

Preparation of Reconstituted HDL Particles : Discoidal reconstituted A-I HDLs were prepared by the cholate dialysis method from egg yolk phosphatidylcholine, unesterified cholesterol, and apo A-I/apo A-II (Matz CE, Jonas A. Micellar complexes of human apolipoprotein A-I with phosphatidycholines and cholesterol prepared from cholate-lipid dispersion. *J.Biol.Chem.1982; 257; 4535-4540*). Egg yolk phosphatidycholine, unesterified cholesterol and sodium cholate were obtained from Sigma and used without further purification. Particle size was measured by nondenaturing gradient gel electrophoresis, and concentration of apo A-I and apo A-II was measured immunoturbidimetrically.

Results : Discoidal reconstituted HDL particles containing either apo A-I (open squares) or apo A-II (closed squares), as the sole protein, were able to inhibit TNFα-induced expression of both arterial and venous endothelial cells VCAM-1. Figure 7a (HUVEC) and 7b (HuAEC) show reconstituted HDL containing apo A-I, as the sole proteins, having a t1/2 max of approximately 3 µMolar, whilst reconstituted HDL containing apo A-II as the sole protein has a give five-fold greater t1/2 max of 15 µMolar.

Conclusion : The therapeutic action of HDL can be mimicked using either apo A-I or apo A-II in reconstituted lipoprotein particle.

C L A I M S :

1. Use of high density lipoprotein and/or a derivative thereof in the manufacture of a medicament for the prevention or treatment of organ dysfunction following haemorrhagic shock.

2. Use according to claim 1 where the medicament is for the treatment of end-stage organ injury or failure.

3. Use according to claim 1 or 2 where the high density lipoprotein or derivative thereof is a peptide or protein derivative of the sequence of apo A-I or apo A-II, or a peptide or protein derivative functionally homologous to the active portions of apo A-I or apo A-II.

4. Use according to claim 1 or 2 where the high density lipoprotein is reconstituted HDL.

5. Use according to claim 1 or 2 where the high density lipoprotein is native HDL.

6. A method of treatment of organ dysfunction following haemorrhagic shock in a human patient which comprises the step of administering to the patient reconstituted high density lipoprotein and/or a derivative thereof in pharmaceutically acceptable form.

FIGURE 1

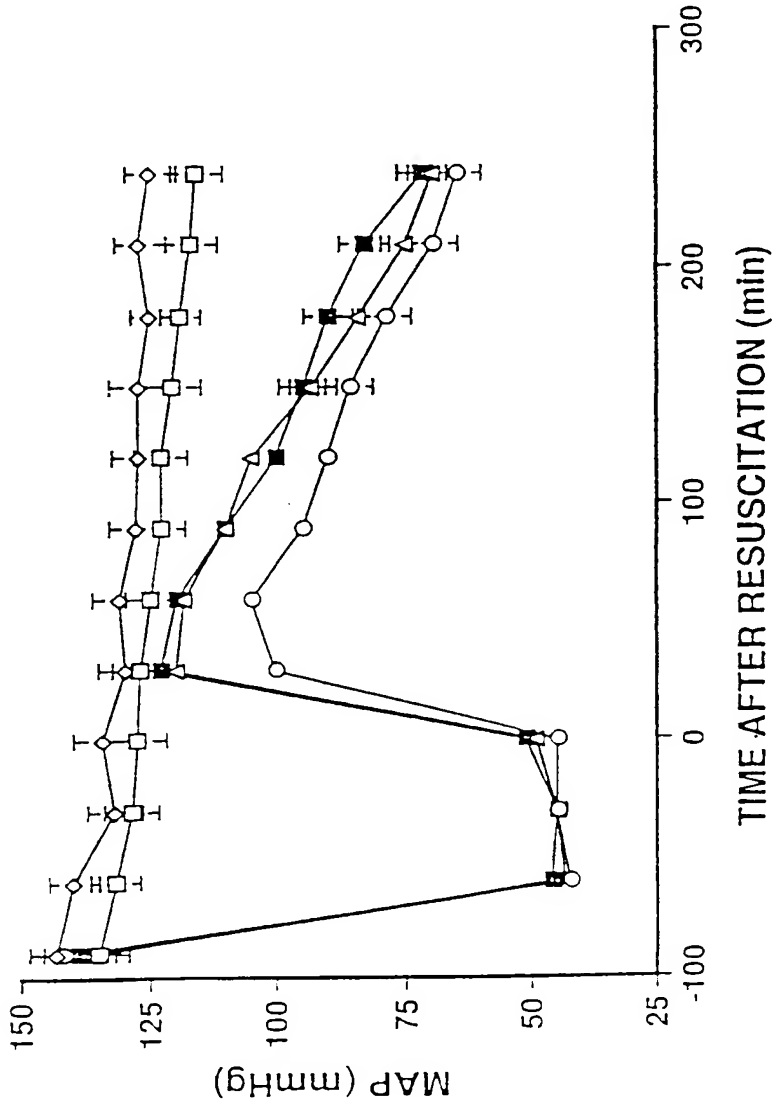

MAP (mmHg)
150
125
100
75
50
25
-100
0
100
200
300
TIME AFTER RESUSCITATION (min)

FIGURE 1A Heart rate (beats per min) in all experimental groups before hemorrhage (Baseline) and 1.2.3, and 4 h after resuscitation.

Group	n	Baseline	Resuscitation Time (h)			
			1	2	3	4
1. SHAM	9	381 ± 15	369 ± 11	385 ± 13	384 ± 13	377 ± 14
2. SHAM-recHDL	4	416 ± 10	387 ± 12	376 ± 8	393 ± 6	380 ± 18
3. HS	9	386 ± 11	417 ± 10	423 ± 20	398 ± 24	372 ± 39
4. HS-recHDL	9	364 ± 13	386 ± 13	407 ± 10	391 ± 14	378 ± 21
5. HS-nHDL	7	388 ± 11	381 ± 15	369 ± 8	356 ± 12	340 ±12

Group 1: Rats were subjected to the surgical procedure without causing a hemorrhage and treated with vehicle for HDLs (saline, 1ml/kg i.v. bolus followed by an infusion of 1.5 ml/kg/h i.v.; n=9); **Group 2:** Rats were subjected to the same surgical procedure as group 1, but were treated with recHDLs (80mg/kg i.v. bolus injection, followed by an infusion of saline 1.5ml/kg/h i.v., n=4); **Group 3:** Rats were subjected to a hemorrhage for 1.5 h and upon resuscitation with the shed blood were given an infusion of saline (1.5ml/kg/h i.v.., n=9); **Group 4:** Rats were subjected to the same procedure as Group 3 but treated with recHDLs (80mg/kg i.v.bolus injection, followed by an infusion of saline 1.5ml./kg/h i.v., n=9); **Group 5:** Rats were treated in the same way as Group 4, but were given nHDLs instead of recHDLs prior to resuscitation. There were no significant differences in the heart rates between any of the experimental groups.

FIGURE 2A

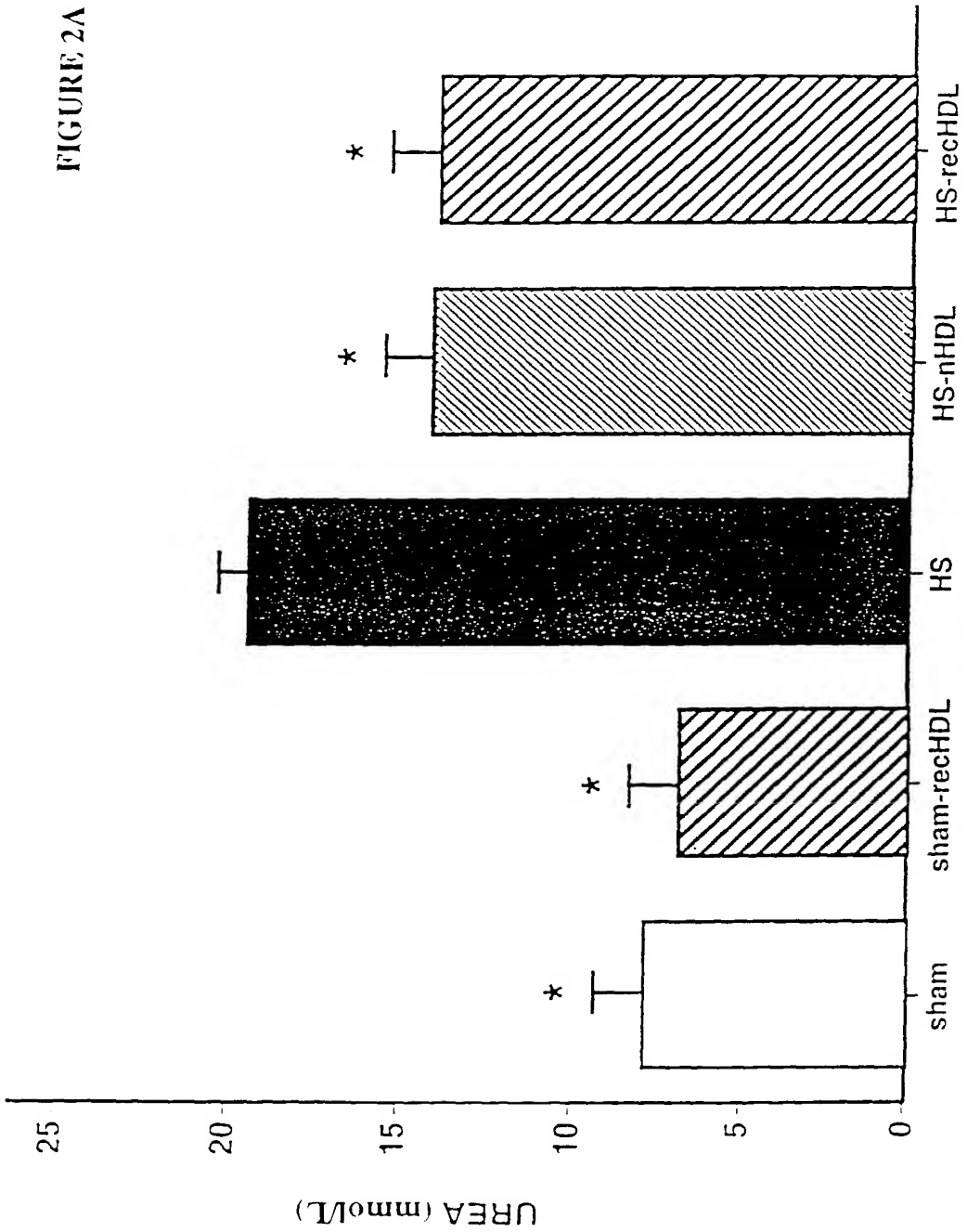


FIGURE 2B

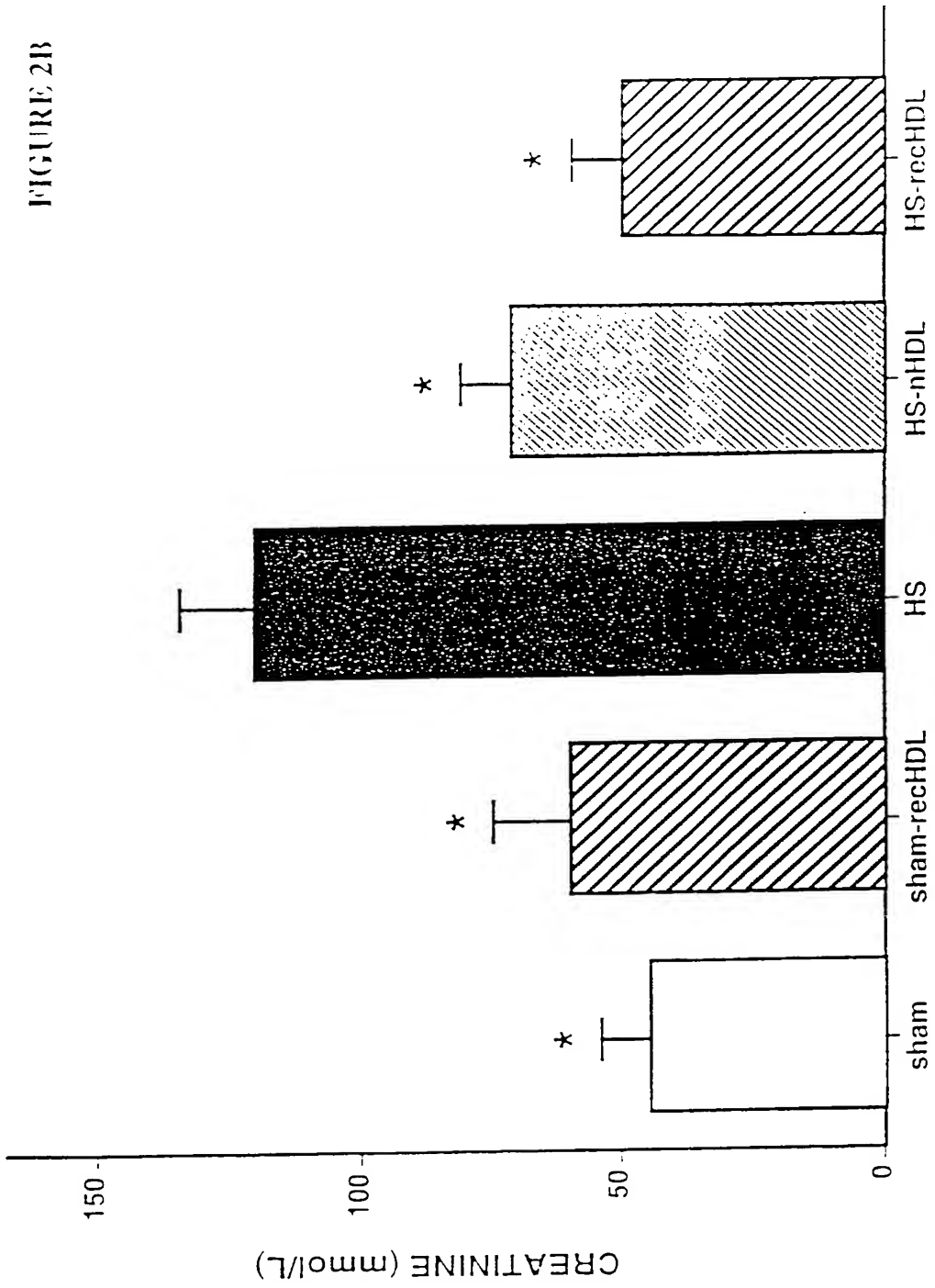

CREATININE (mmol/L)
0
50
100
150
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*
*

FIGURE 2C

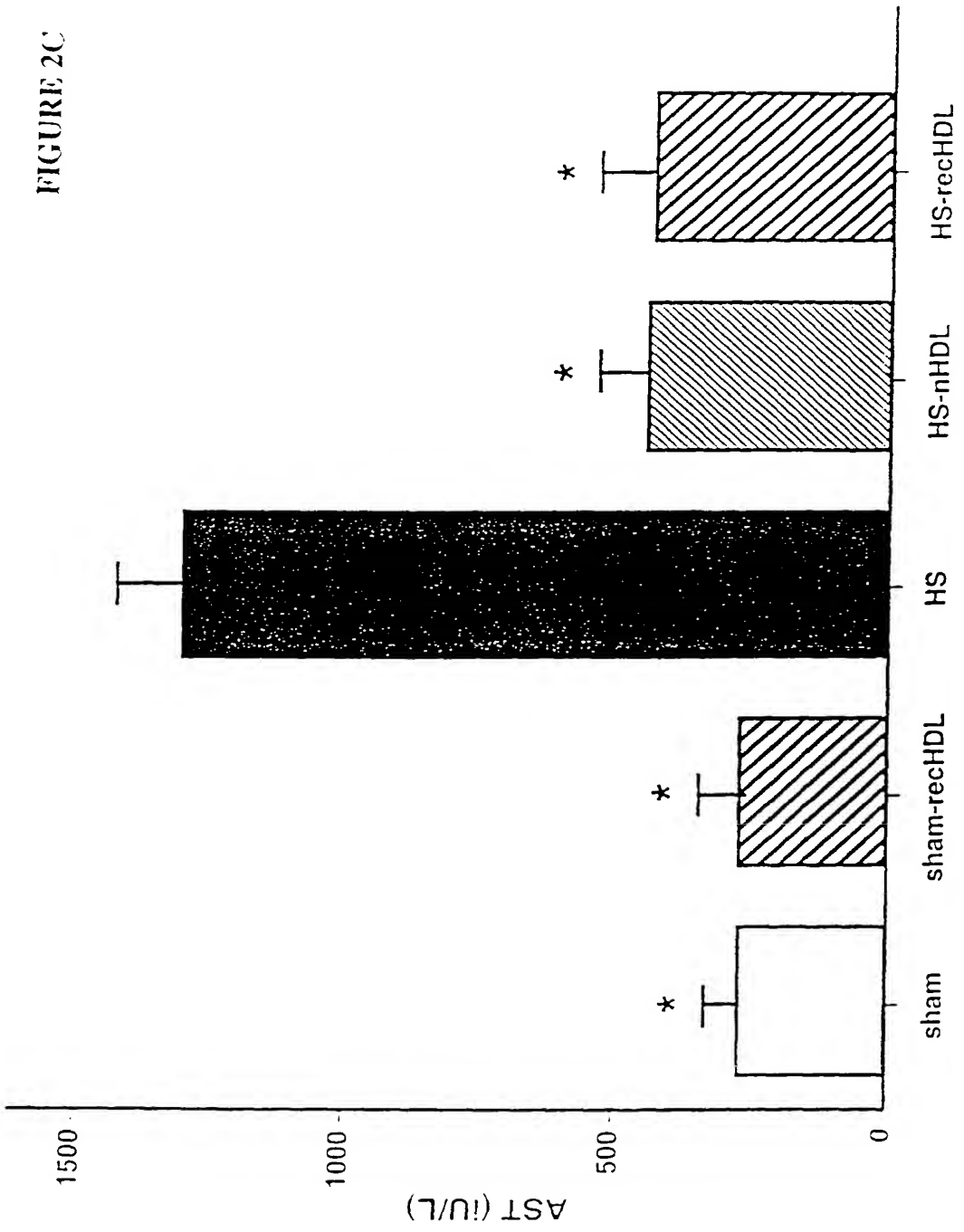


FIGURE 2D

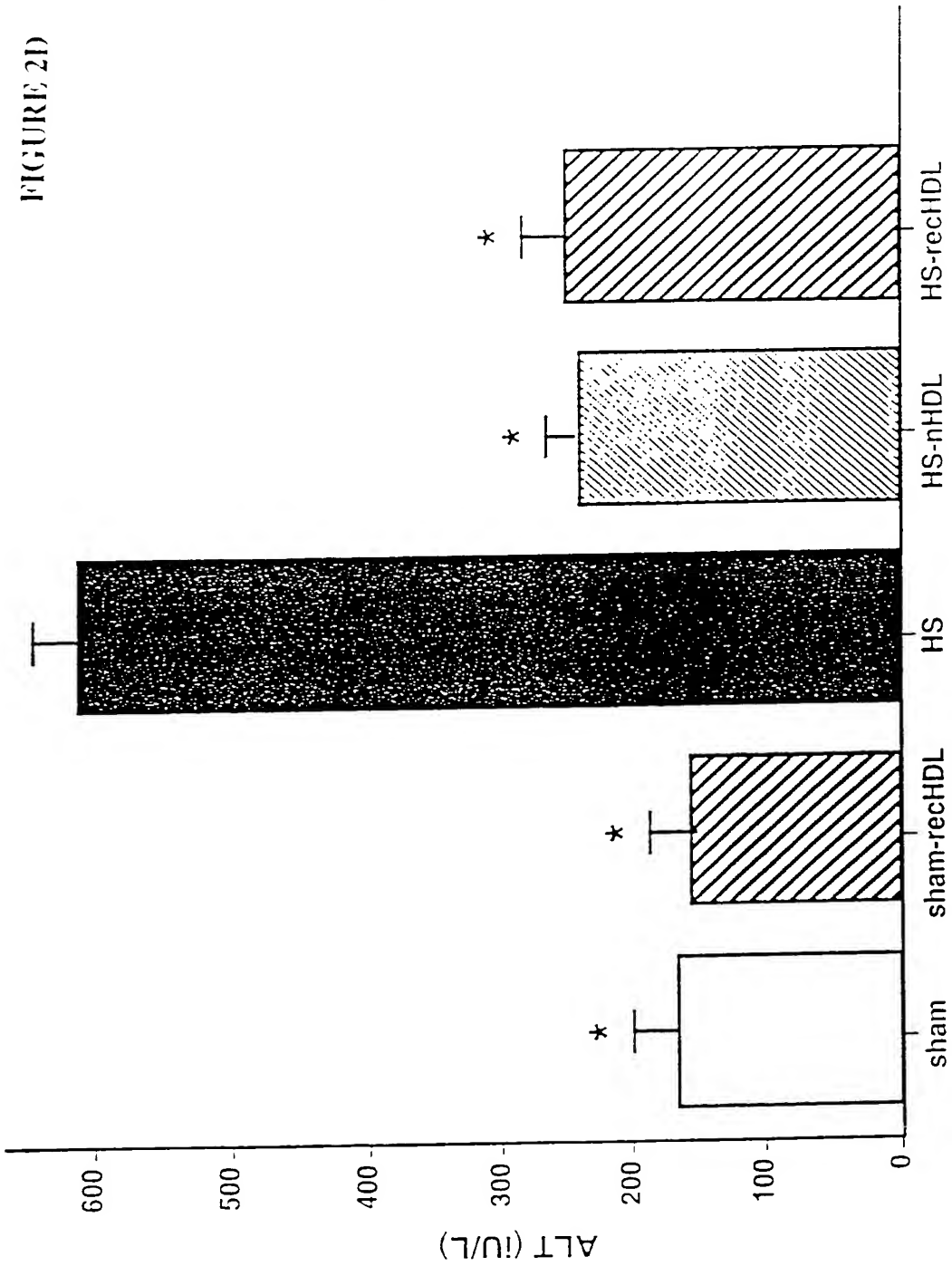

ALT (iU/L)
0
100
200
300
400
500
600
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*

FIGURE 2E

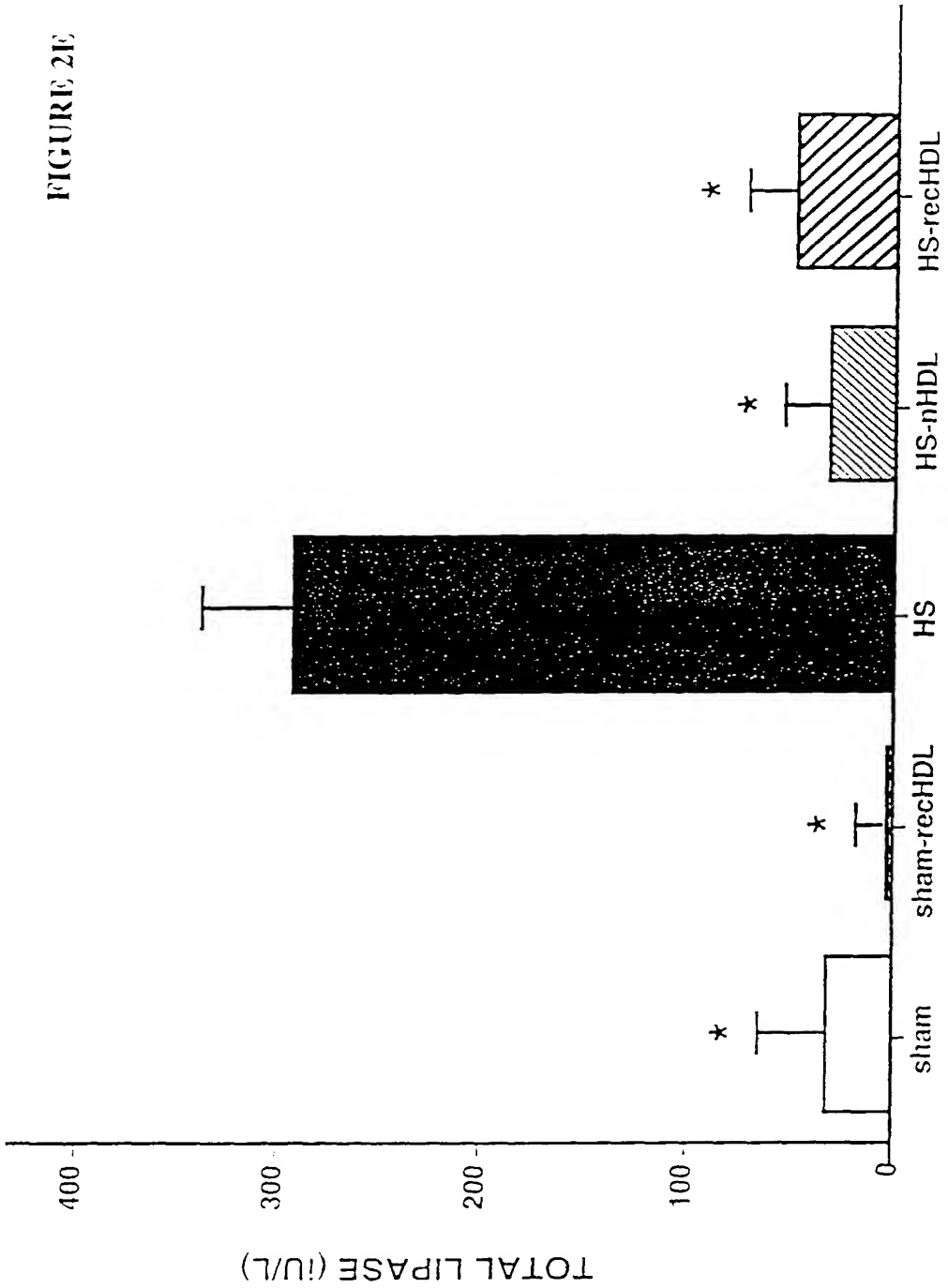

TOTAL LIPASE (iU/L)
400
300
200
100
0
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*
*

FIGURE 2F

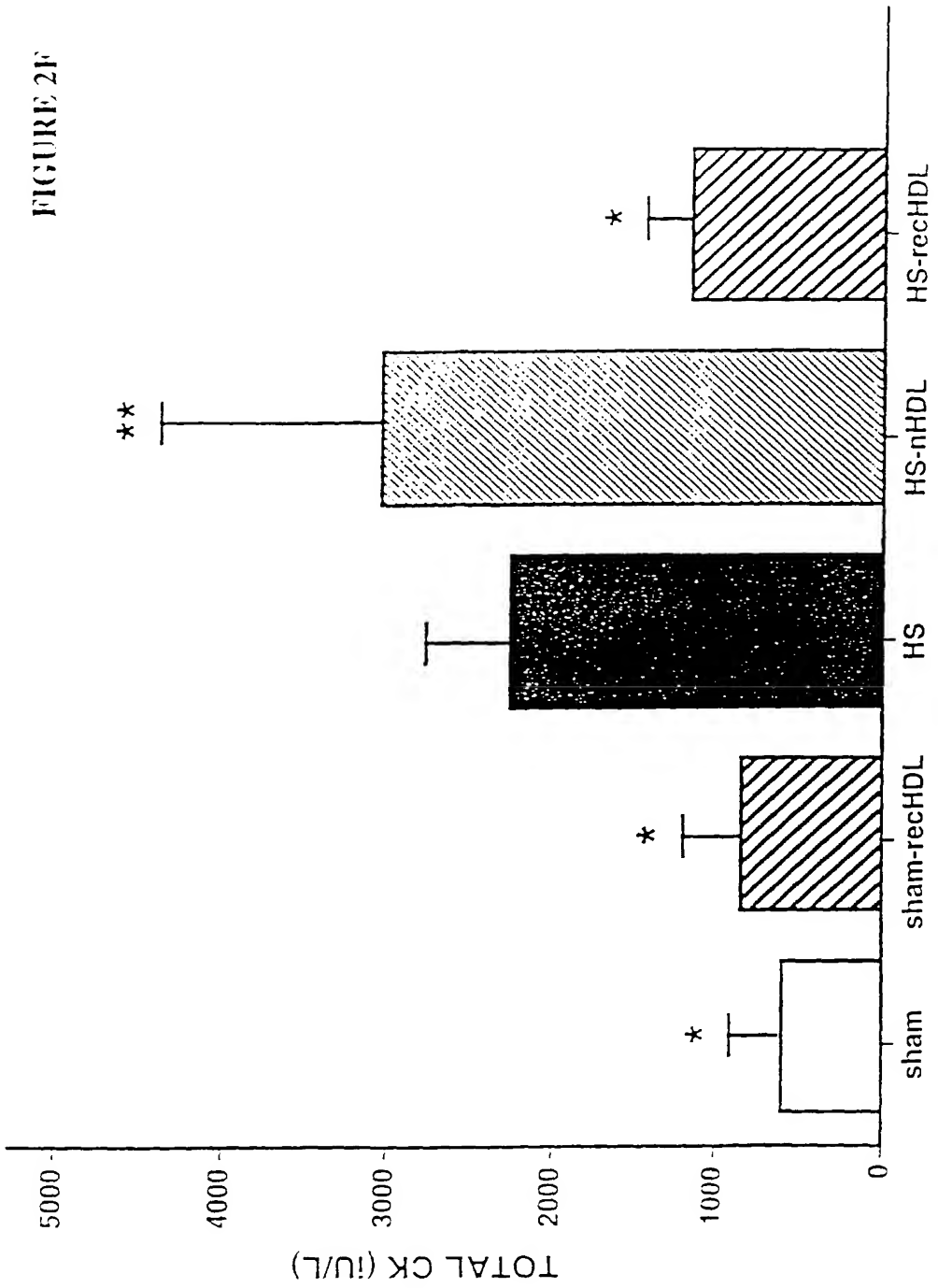

TOTAL CK (iU/L)
0
1000
2000
3000
4000
5000
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
**
*

FIGURE 3

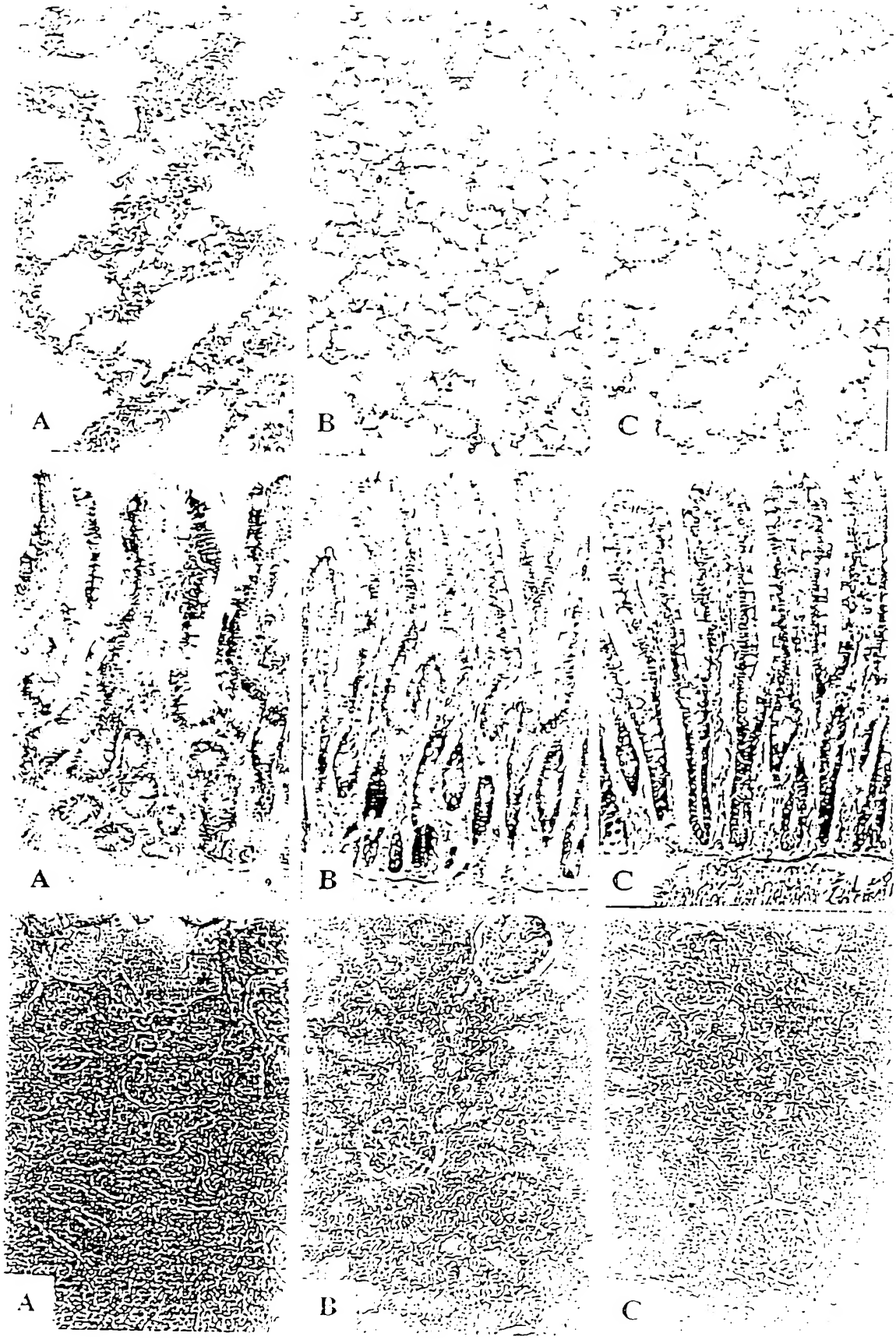


FIGURE 4A

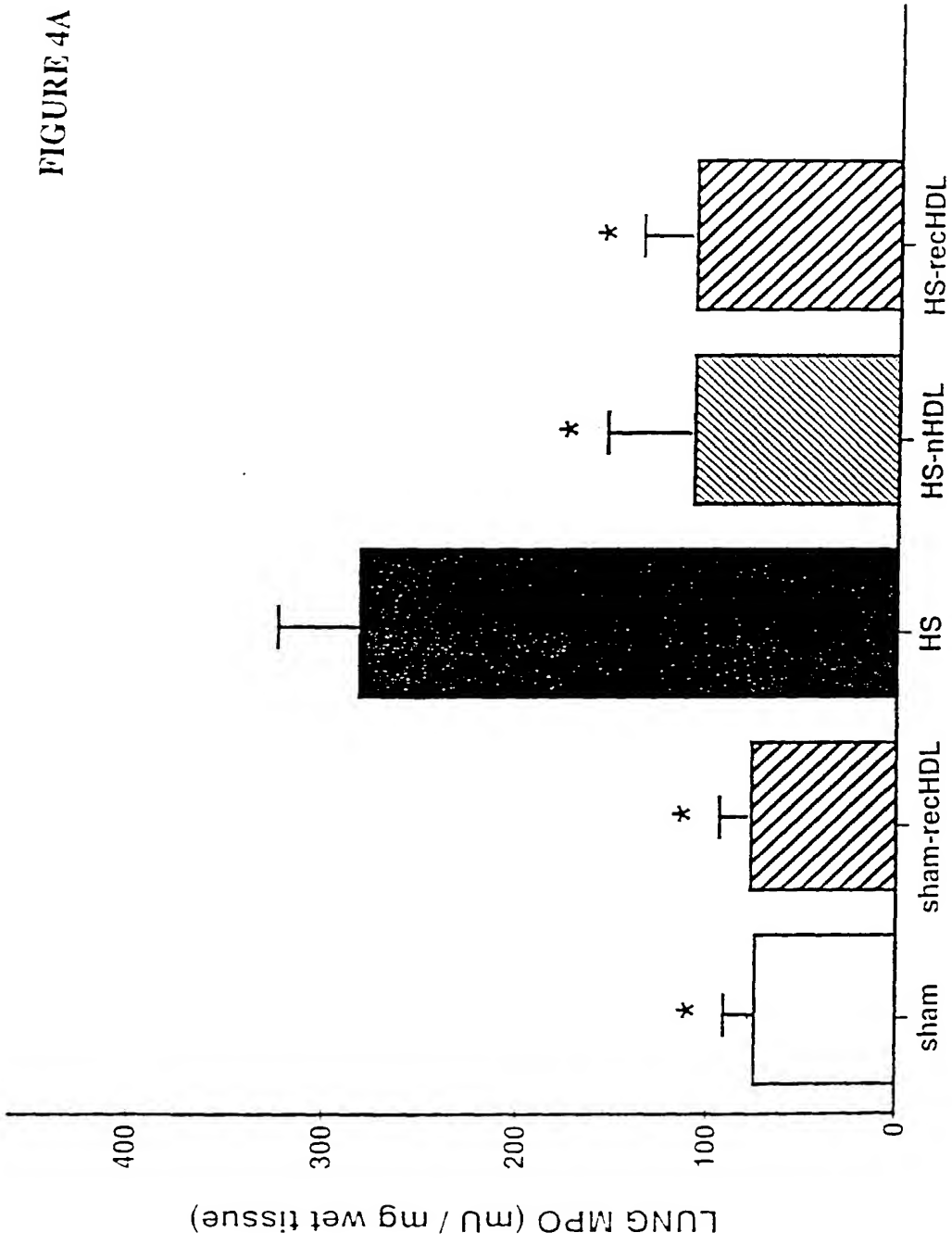

LUNG MPO (mU / mg wet tissue)
0
100
200
300
400
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*

FIGURE 4B

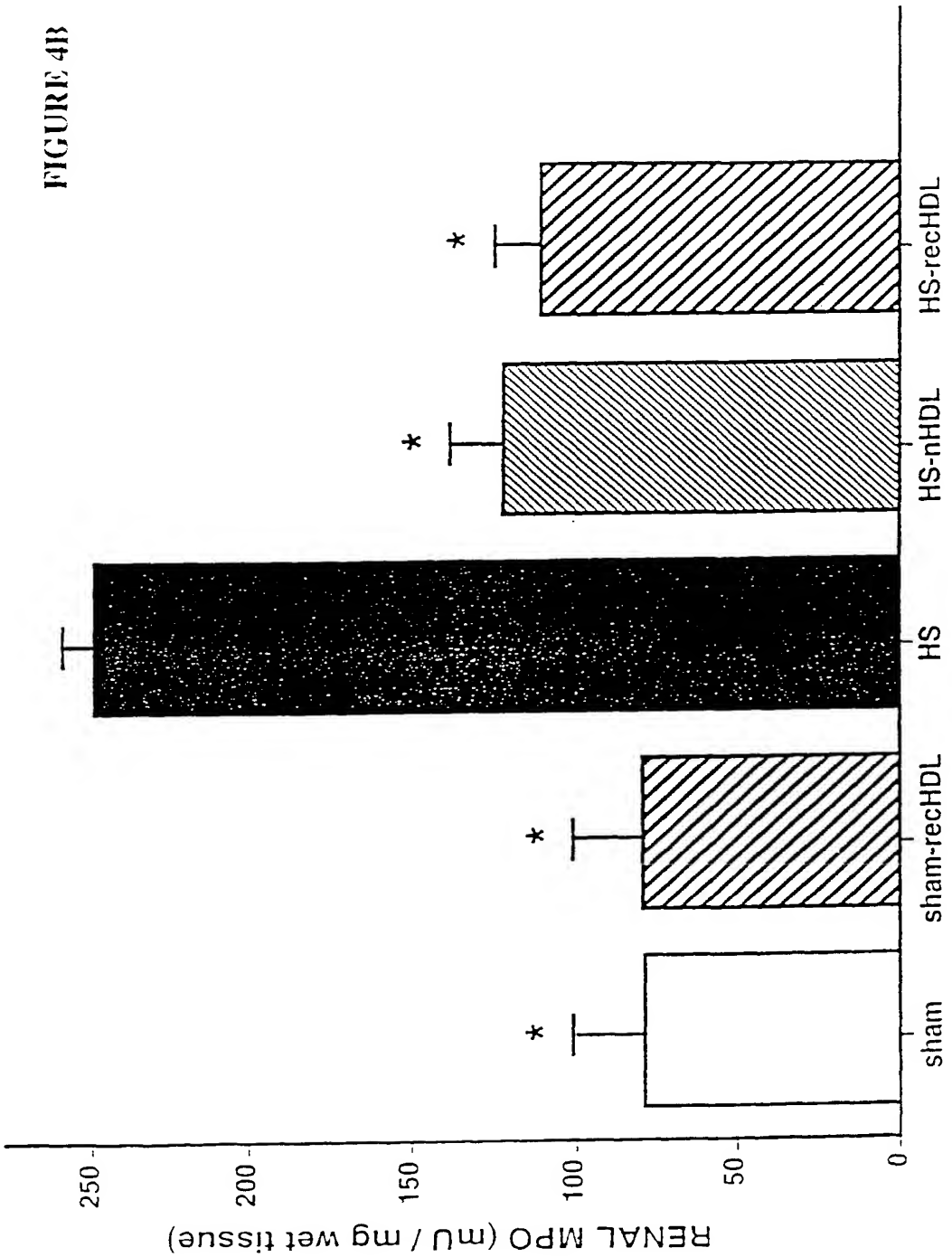

RENAL MPO (mU / mg wet tissue)
250
200
150
100
50
0
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*
*

FIGURE 5A

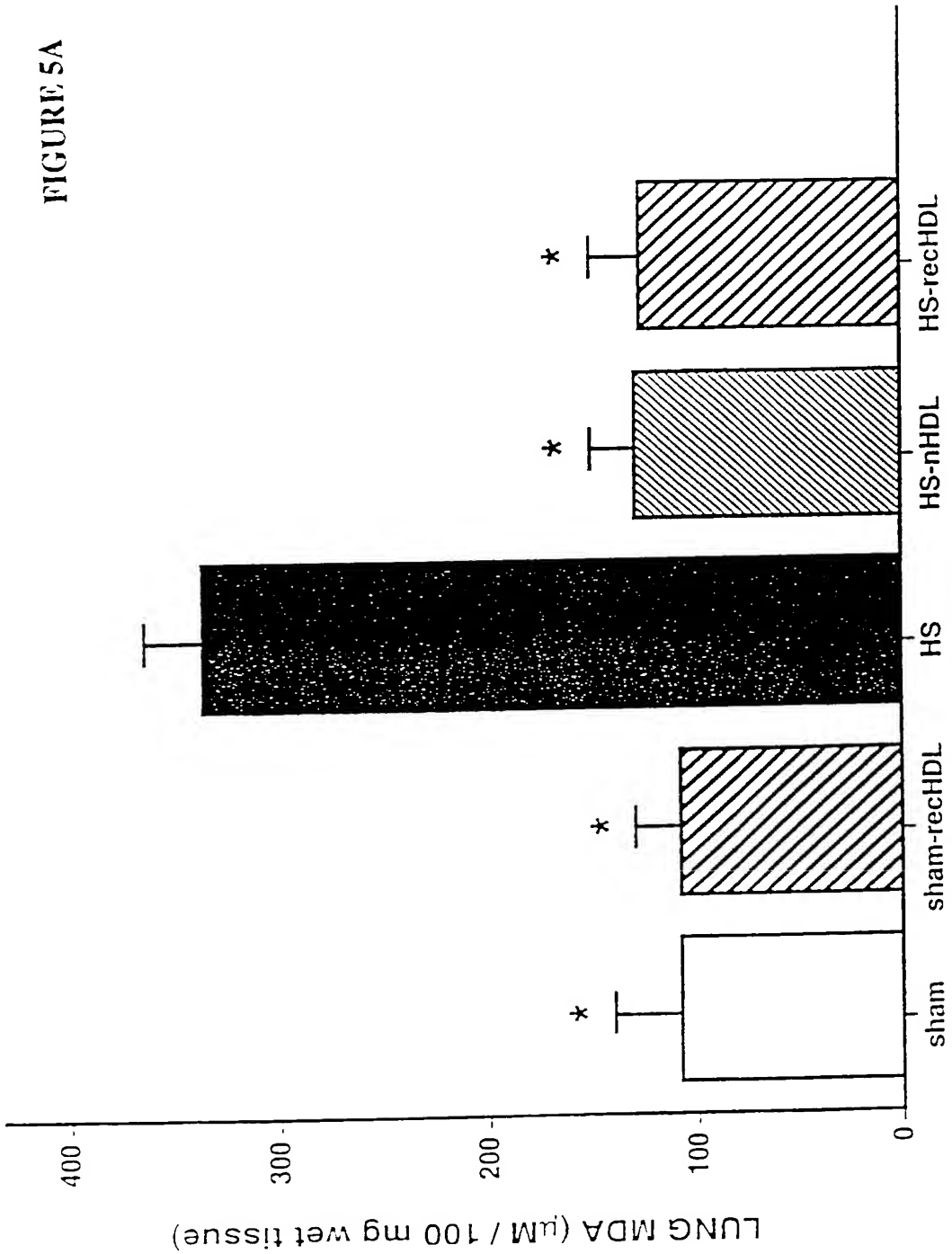

LUNG MDA (μM / 100 mg wet tissue)
0
100
200
300
400
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*

FIGURE 5B

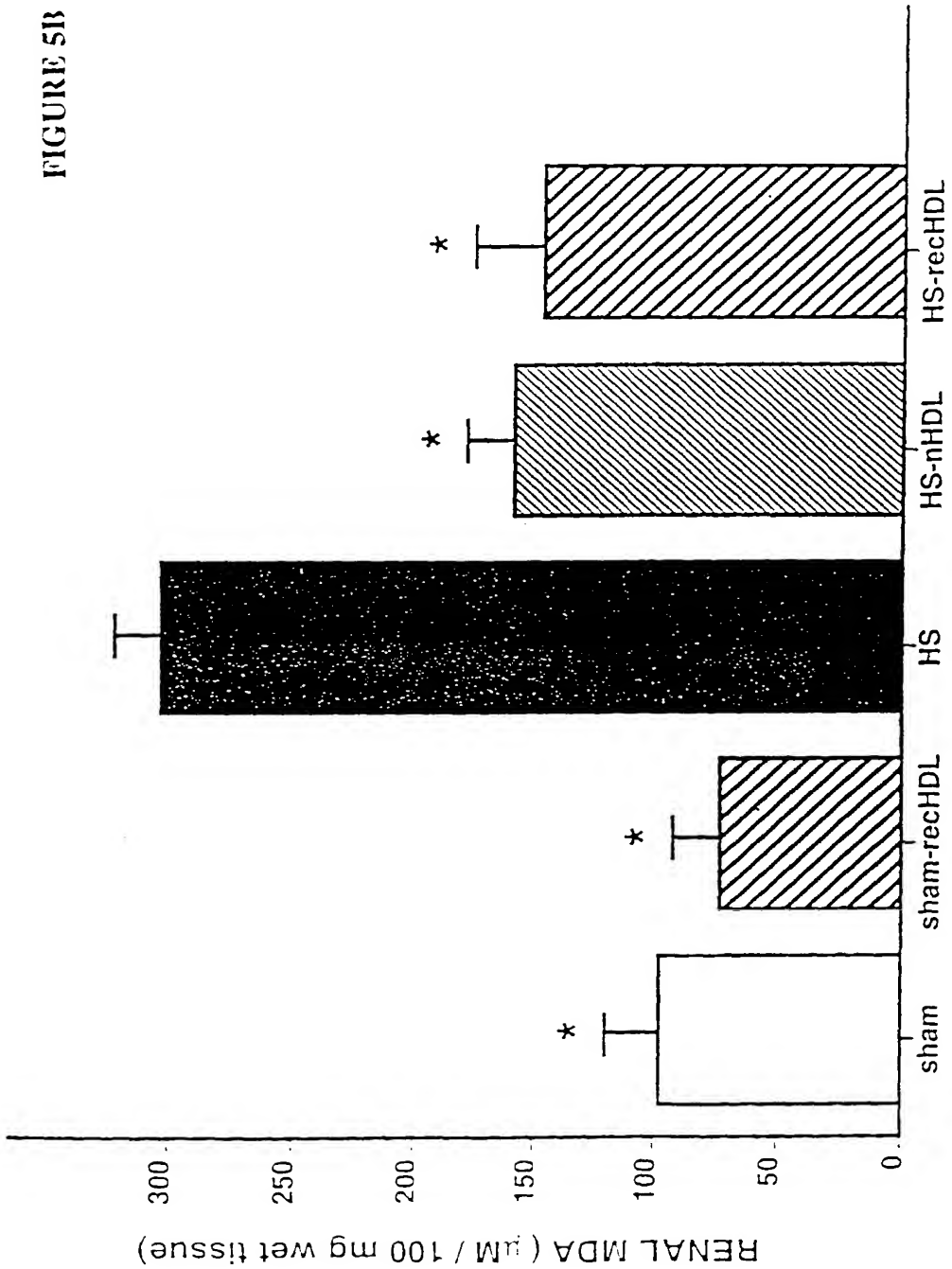

RENAL MDA (μM / 100 mg wet tissue)
0
50
100
150
200
250
300
sham
sham-recHDL
HS
HS-nHDL
HS-recHDL
*
*
*
*

FIGURE 6

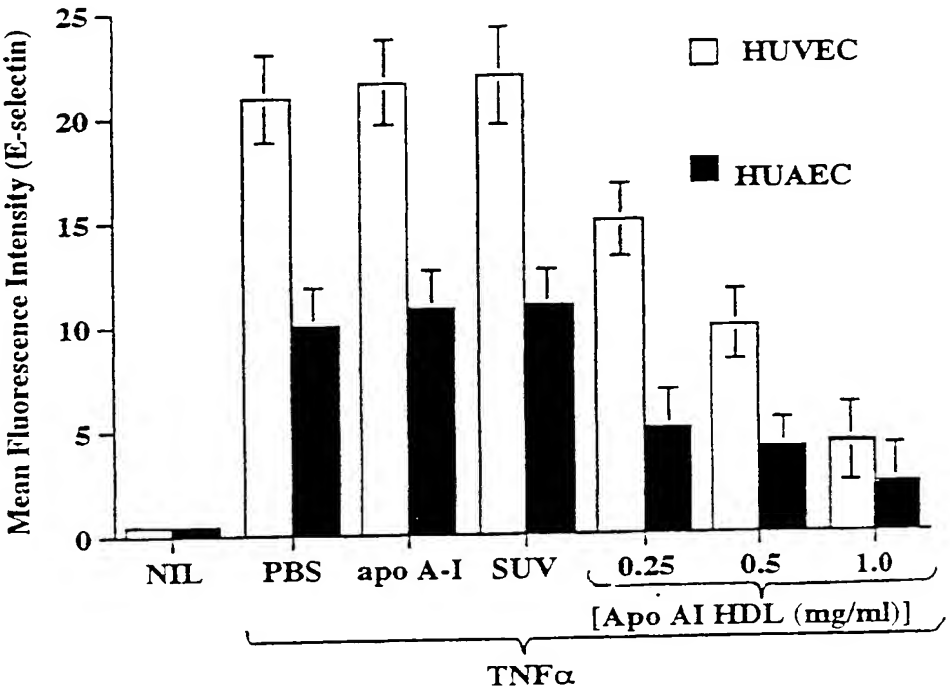

Mean Fluorescence Intensity (E-selectin)
25
20
15
10
5
0
HUVEC
HUAEC
NIL
PBS
apo A-I
SUV
0.25
0.5
1.0
[Apo AI HDL (mg/ml)]
TNFα

FIGURE 7A

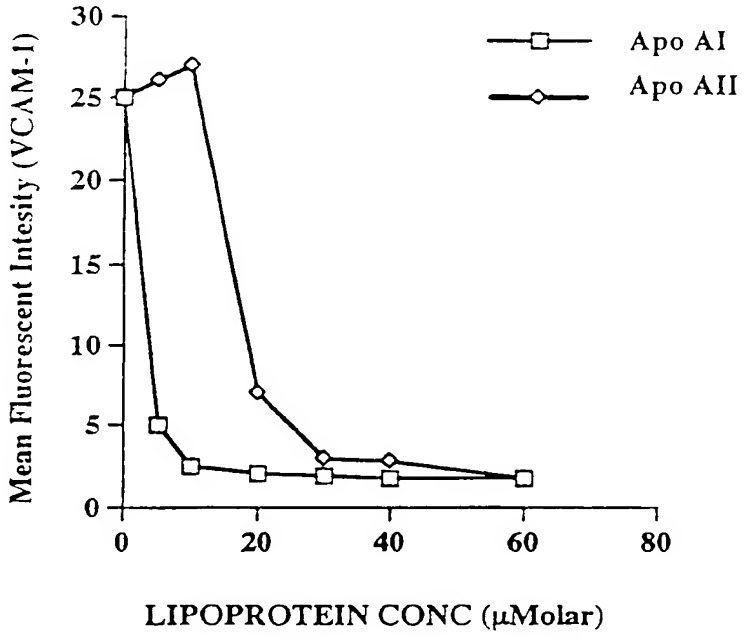
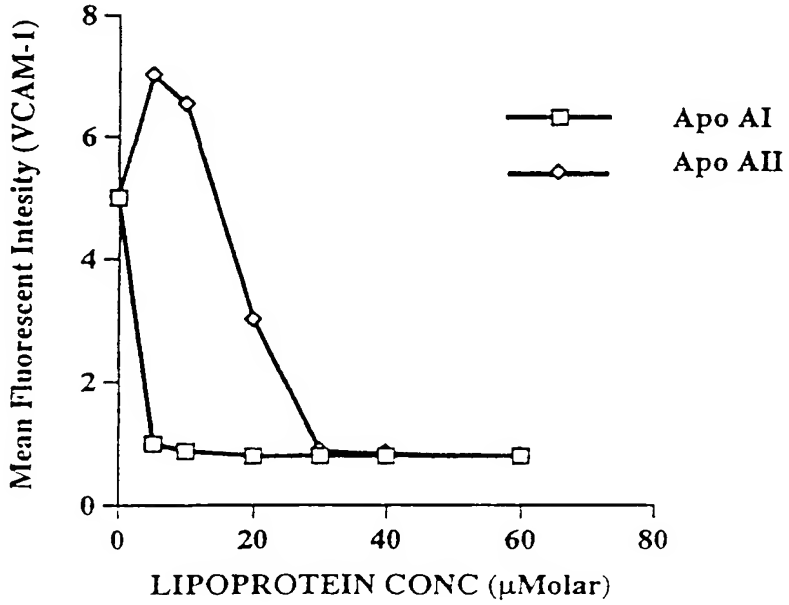


FIGURE 7B



INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 00/03182

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61P39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 780 128 A (HOECHST AG) 25 June 1997 (1997-06-25) *see in particular claims 1,4,5,9; page 3, lines 4-9; example 5 * ---	1-6
A	PATENT ABSTRACTS OF JAPAN vol. 1998, no. 05, 30 April 1998 (1998-04-30) & JP 10 025248 A (CHEMO SERO THERAPEUT RES INST), 27 January 1998 (1998-01-27) abstract ---	1-6
A	WO 97 06822 A (PROTEIN DESIGN LABS INC ;BOEHRINGER MANNHEIM GMBH (DE); HASELBECK) 27 February 1997 (1997-02-27) *see in particular page 1, line 5 - page page 3, line 12; claims 1,10,22* ---	1-6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	18/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Isert, B

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int[illegible] Application No
PCT/GB 00/03182

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
A	COCKERILL ET AL.: "High-density lipoproteins differentially modulate cytokine-induced expression of E-selectin and cyclooxygenase-2 " ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, vol. 19, April 1999 (1999-04), pages 910-917, XP000944093 *see the abstract; p.915, last paragraph of the discussion *	1-6

1

INTERNATION[redacted] SEARCH REPORT

.nformation on patent family members

Inte[redacted]Application No
PCT/GB 00/03182

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0780128 A	25-06-1997	DE 19547648 A	26-06-1997
		JP 9188658 A	22-07-1997
		US 5780592 A	14-07-1998
JP 10025248 A	27-01-1998	NONE	
WO 9706822 A	27-02-1997	AU 6773596 A	12-03-1997
		CA 2229140 A	27-02-1997
		CZ 9800454 A	13-01-1999
		EP 0868197 A	07-10-1998
		HU 9901673 A	30-08-1999
		JP 10510553 T	13-10-1998
		PL 325012 A	06-07-1998